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| (54) Title: BAU, A BIN1 INTERACTING PROTEIN, AND USES THEREFOR (57) Abstract A murine cDNA clone encoding a BIN1-Associated U1-specific protein (Bau) are provided. Also provided are methods of using the nucleic acid sequences, polypeptides, and antibodies directed against same in the diagnosis and treatment of cancers, hyperplastic disease states, or degenerative diseases. | | |

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BAU, A BIN1 INTERACTING PROTEIN, AND USES THEREFOR

Field of the Invention

This invention relates generally to cancer diagnosis and therapy, and more specifically, to cancers associated with activation of the Myc oncoprotein and/or loss of Bin1 tumor suppression.

Background of the Invention

There is a significant need for effective therapies against many types of cancers, especially carcinoma, which is often untreatable in its advanced states. Cell regulation by the Myc oncoprotein offers an attractive focus for therapeutic development. Myc is deregulated in a wide variety of cancers, including most carcinomas, through genetic and epigenetic mechanisms [M.D. Cole, Ann. Rev. Genet., 20:361-384 (1986)]. Thus, Myc-regulated mechanisms are logical targets for developing novel and broadly applicable therapeutic strategies.

Myc acts at the intersection of pathways that control cell division, differentiation, and apoptosis. In normal cells, Myc is rapidly induced following mitogenic stimulation and remains elevated throughout the cell cycle [Evan and Littlewood, Curr Opin Genet Dev, 3: 44-49 (1993)]. Induction of Myc is sufficient to drive cell proliferation [M. Eilers, et al., Nature, 340: 66-8 (1989)], while inhibition of Myc can block mitogenic signals and facilitate cell differentiation [R. Heikkila, et al., Nature, 328: 445-448 (1987); J.T. Holt, et al., Mol Cell Biol, 8: 963-973 (1988); K. D. Hanson, et al., Mol Cell Biol, 14: 5748-5755 (1994)]. Significantly, Myc can induce apoptosis [D.S. Askew, et al., Oncogene, 6: 1915-1922 (1991); G.I. Evan, et al., Cell, 69: 119-128 (1992)], if its expression is uncoupled from the orchestration of other cell cycle regulatory events [G. I. Evan, et al., cited above]. Clinical evidence

indicates that loss of the apoptotic response is associated with malignant conversion. Therefore, reactivation or derepression of this response would be desirable. Myc-activated death in epithelial cells (the precursor cell type to carcinoma) is p53-independent [D. Sakamuro, et al., Oncogene, 11: 2411-2418 (1995)], a useful feature because p53 function is often lost in carcinoma [Levine, Ann Rev Biochem, 62: 623-651 (1993)]. Thus, using Myc-activated death mechanisms is attractive, since the tumor cell could be attacked without regard to its p53 status.

Bin1 is a 451 amino acid Myc-interacting nuclear phosphoprotein [D. Sakamuro, et al., Nature Genet, 14: 69-77 (1996)], which has been implicated in the mechanism by which Myc induces apoptosis. Bin1 has several features of a tumor suppressor that is lost in breast and prostate carcinoma, where loss of apoptotic potential is tantamount to malignant conversion. First, Bin1 suppresses malignant cell transformation by Myc, but also by adenovirus E1A and by mutant p53, which act by Myc-independent mechanisms. Second, while normally ubiquitously expressed, Bin1 is frequently missing in breast and prostate carcinoma cell lines and primary tumors. Third, these deficits in expression appear to be functionally significant, because ectopic expression of Bin1 inhibits the growth of tumor cells which lack endogenous Bin1. Fourth, the N-terminal region of Bin1, termed the BAR domain, is closely related to a breast cancer-associated autoimmune antigen (amphiphysin) and a negative regulator of the yeast cell cycle (RVS167) [D. Sakamuro, et al., cited above.]. Finally, the human Bin1 gene maps to chromosome 2q14 [D. Negorev, et al., Genomics, 33: 329-331 (1996)], within a mid-2q region that is among the more frequently deleted loci in

metastatic prostate cancers [M. L. Cher, et al., Canc Res, 56: 3091-3102 (1996)].

The murine and human BIN1 sequences, provided herein as Figs. 2 and 3, are described in more detail in WO 96/34627. Of particular interest is the unique-1 (U1) region (located between aa 225-250) [D. Sakamuro et al, cited above; R. Wechsler-Reya, et al., Cancer Res, 57:3258-3263 (1997)], which mediates efficient cell growth inhibition through both Myc-dependent and Myc-independent mechanisms. U1 is encoded by exon 9 in the human Bin1 gene, located adjacent to the alternatively spliced exon 10.

There remains a need in the art for compositions and methods of regulating a deregulated Myc protein and of treating and diagnosing cancers associated with the Myc oncoprotein and/or undesirably low Bin1 levels.

Summary of the Invention

In one aspect, the present invention provides a murine cDNA clone of a BIN1-Associated U1-specific protein (Bau) SEQ ID NO:1 and the polypeptide encoded thereby, SEQ ID NO:2.

In yet another aspect, the present invention provides a vector comprising a mammalian nucleic acid sequence encoding a BIN1 protein and a host cell transformed by such a vector. Alternatively, this vector may be used in gene therapy applications.

In still another aspect, the invention provides an oligonucleotide probe comprising a nucleic acid sequence as defined herein. Also provided is an antibody raised against a Bau protein or peptide thereof.

In yet a further aspect, the present invention provides a diagnostic reagent for cancers, involving Myc, inappropriately high Bau levels, or inappropriately low

Bin levels, comprising an oligonucleotide probe or an antibody of the invention.

Further provided is a therapeutic reagent comprising a polypeptide, anti-idiotypic antibody, or gene therapy
5 vector of the invention.

Still another aspect of the invention provides a method of treating cancers involving Myc by administering a therapeutic reagent of the invention.

Other aspects and advantages of the present
10 invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Figs. 1A-1B is a murine Bau cDNA sequence [SEQ ID NO:1] and the murine Bau polypeptide encoded thereby [SEQ
15 ID NO:2].

Fig. 2 is a murine cDNA sequence [SEQ ID NO:3] encoding a BIN1 polypeptide [SEQ ID NO:4].

Figs. 3A-3C is a human cDNA sequence [SEQ ID NO:5] encoding a BIN1 polypeptide [SEQ ID NO:6].

20 Fig. 4 is a bar chart illustrating Bau suppression of malignant transformation of rat embryo fibroblasts by the adenovirus Ela oncoprotein.

Detailed Description of the Invention

The present invention provides novel, isolated,
25 nucleic acid Bau sequences which encode novel proteins which interact with BIN1 and bind thereto, fragments of these sequences and antibodies developed thereto. As described above briefly, BIN1 is a protein that interacts with the functionally critical Myc box regions at the N-
30 terminus of the Myc oncoprotein and which is associated with tumor suppression and apoptosis. BIN1 is described in more detail in the International Patent Application which published as WO 96/34627 on November 7, 1996 and US

Patent No. 5,605,830, as well as in co-owned, co-pending US Patent Application No. 08/652,972, which are incorporated herein by reference.

The Bau nucleic acid sequences, amino acid sequences and antibodies of the invention are useful in the detection, diagnosis and treatment of cancers or other disorders associated with inappropriate BIN1 levels and/or deregulation, deficiency or amplification of the c-Myc oncogenes. These aspects of the invention are discussed in more detail below.

I. Nucleic Acid Sequences

The present invention provides mammalian nucleic acid sequences encoding a 293 amino acid polypeptide, termed herein Bau. The nucleic acid sequences of this invention are isolated from cellular materials with which they are naturally associated.

The Bau cDNA was isolated in a yeast two hybrid screen for polypeptides that could specifically associate with the U1 domain (aa 225-250) of the human BIN1 protein [SEQ ID NO:6]. The U1 region is a functionally important region of BIN1 and has a role in protein-protein interaction. U1 contains several amino acid sequence motifs which are found in regulators of the cell cycle and chromosome function. Two ~10 residue motifs show relatedness to the yeast proteins p93dis1 [Nabeshima et al, Genes Dev., 9:1572-1585 (1995)] and RED1 [Thompson and Roeder, Mol. Gen. Genet., 218:293-301 (1989)], which are involved in chromosome segregation. An additional sequence motif is shared with a region of the SV40 virus T antigen protein (aa 5-35) which is implicated in its cellular immortalization activity [Conzen and Cole, Oncogene, 11:2295-2302 (1995)]. Notably, the BIN-T antigen similarity is of the form D Ψ LXGXE [SEQ ID NO:7] (the greek psi represents a hydrophobic amino acid),

which is reminiscent of the (D)LXCXE [SEQ ID NO:8] motif which mediates protein-protein interactions with the retinoblastoma (Rb) protein, an important regulator of the G1 phase of the cell cycle. A connection to Rb is intriguing, because an Rb-sized ~110 kD protein(s) is specifically coimmunoprecipitated with BIN1 from cell lysates by anti-BIN1 monoclonal antibodies. Further, in cell transformation experiments, U1 deletion reduced the activity of BIN1 to inhibit the oncogenic activity of MYC, and abolished the ability of BIN1 to inhibit the oncogenic activity of the adenovirus E1A oncoprotein, which is functionally related to MYC and which must inactivate Rb to transform cells. The ability of the 293 aa Bau polypeptide to inhibit E1A-mediated cell transformation, similar to BIN1, indicates its direct role in regulating U1. Taken together, the data indicates that Bau possesses anti-oncogenic activity, possibly related to apoptosis. Furthermore, the data suggests that Bau influences or mediates Bin1 activity through interactions with U1.

Thus, in one embodiment, the invention provides a Bau nucleic acid sequence which is selected from all or part of the murine cDNA clone, SEQ ID NO: 1. However, the present invention is not limited to these nucleic acid sequences.

Given the sequences of SEQ ID NO: 1, one of skill in the art can readily obtain the corresponding anti-sense strands of these cDNA and genomic sequences. Further, using known techniques, one of skill in the art can readily obtain the human sequences corresponding to these cDNA sequences or the corresponding RNA sequences, as desired.

Similarly, the availability of SEQ ID NO: 1 of this invention permits one of skill in the art to obtain other species Bau homologs, by use of the nucleic acid

sequences of this invention as probes in a conventional technique, e.g., polymerase chain reaction. Allelic variants of these sequences within a species (i.e., nucleotide sequences containing some individual

5 nucleotide differences from a more commonly occurring sequence within a species, but which nevertheless encode the same protein), may also be readily obtained given the knowledge of this sequence provided by this invention.

The present invention further encompasses nucleic acid sequences capable of hybridizing under stringent conditions [see, J. Sambrook et al, Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory (1989)] to the sequences of SEQ ID NO: 1, their anti-sense strands, or biologically active fragments thereof.

15 An example of a highly stringent hybridization condition is hybridization at 2XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively, an exemplary highly stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Moderately high stringency

20 conditions may also prove useful, e.g. hybridization in 4XSSC at 55°C, followed by washing in 0.1XSSC at 37°C for an hour. An alternative exemplary moderately high stringency hybridization condition is in 50% formamide, 4XSSC at 30°C.

25 Also encompassed within this invention are fragments of the above-identified nucleic acid sequences. Preferably, such fragments are characterized by encoding a functional fragment of Bau, e.g., the Bin1-binding domain (nucleotides 231 to 674 of SEQ ID NO:1), an

30 epitope, or another fragment characterized by having a desired biological activity. Generally, these oligonucleotide fragments are at least 15 nucleotides in length. However, oligonucleotide fragments of varying sizes may be selected as desired. Such fragments may be

35 used for such purposes as performing the PCR, e.g., on a

biopsied tissue sample. For example, one nucleotide fragment optimal for PCR is the fragment defined by nucleotides 231 to 674 within SEQ ID NO: 1. Other useful fragments may be readily identified by one of skill in the art by resort to conventional techniques, e.g., by computerized motif searching. Examples of such useful fragments include (with reference to SEQ ID NO:1) nt 48 to 527, which encode a coiled-coil domain; nt 312 to 362, which encode a signature motif; nt 294 to 356, which encode a cystatin motif; and nt 540 to 554, which encode a polyasparagine region.

The nucleotide sequences of the invention may be isolated by conventional uses of polymerase chain reaction or cloning techniques such as those described in obtaining the murine sequences, described below. Alternatively, these sequences may be constructed using conventional genetic engineering or chemical synthesis techniques.

According to the invention, the nucleic acid sequences [SEQ ID NO: 1] may be modified. Utilizing the sequence data in these figures and in the sequence listing, it is within the skill of the art to obtain other polynucleotide sequences encoding the proteins of the invention. Such modifications at the nucleic acid level include, for example, modifications to the nucleotide sequences which are silent or which change the amino acids, e.g. to improve expression or secretion. Also included are allelic variations, caused by the natural degeneracy of the genetic code.

Also encompassed by the present invention are mutants of the Bau gene provided herein. Such mutants include amino terminal, carboxy terminal or internal deletions which are useful as dominant inhibitor genes. Such a truncated, or deletion, mutant may be expressed for the purpose of inhibiting the activity of the full-

length or wild-type gene. These nucleic acid sequences are useful for a variety of diagnostic and therapeutic uses. Advantageously, the nucleic acid sequences are useful in the development of diagnostic probes and antisense probes for use in the detection and diagnosis of conditions characterized by inappropriate BIN1 levels, which may be associated with deregulation or amplification of c-MYC. The nucleic acid sequences of this invention are also useful in the production of mammalian, and particularly, murine and human BIN1 proteins.

II. Protein Sequences

The present invention also provides mammalian Bau polypeptides or proteins. These proteins are free from association with other contaminating proteins or materials with which they are found in nature. In one embodiment, the invention provides a murine Bau [SEQ ID NO:2] polypeptide of 293 amino acids having a predicted molecular weight (MW) 34,137.

Further encompassed by this invention are fragments of the Bau polypeptides. Such fragments are desirably characterized by having Bau biological activity, including, e.g., the ability to interact with BIN1. An example of such a fragment is aa 62 to 209, which encompasses the Bin1-binding domain. Other useful fragments may be designed or obtained in any desired length, including as small as about 8 amino acids in length. Such a fragment may represent an epitope of the protein. Further, one of skill in the art can readily identify other functional fragments, e.g., by computer motif analysis. Examples of such useful fragments include (with reference to SEQ ID NO:2), aa 1 to 160 which is a coiled-coil domain; aa 89 to 105, which is a signature motif; aa 83 to 103, which is a cystatin motif; and aa 165 to 169, which is a polyasparagine region.

Also included in the invention are analogs, or modified versions, of the proteins provided herein. Typically, such analogs differ by only one to four codon changes. Examples include polypeptides with minor amino acid variations from the illustrated amino acid sequences of Bau (Fig. 1; SEQ ID NO:2); in particular, conservative amino acid replacements. Conservative replacements are those that take place within a family of amino acids that are related in their side chains and chemical properties. Also provided are homologs of Bau. Based on the sequence information provided herein, one of skill in the art can readily obtain Bau from other mammalian species. Such analogs and homologs are typically at least about 85% homologous with SEQ ID NO: 2, and more desirably, at least about 90% homologous, as determined by sequence comparison algorithms such as WU-BLAST2 (Washington University BLAST).

Additionally, the Bau proteins [SEQ ID NO:2] of the invention may be modified, for example, by truncation at the amino or carboxy termini, by elimination or substitution of one or more amino acids, or by any number of now conventional techniques to improve production thereof, to enhance protein stability or other characteristics, e.g. binding activity or bioavailability, or to confer some other desired property upon the protein.

III. Expression

A. In Vitro

To produce recombinant Bau proteins of this invention, the DNA sequences of the invention are inserted into a suitable expression system. Desirably, a recombinant molecule or vector is constructed in which the polynucleotide sequence encoding Bau is operably linked to a heterologous expression control sequence

permitting expression of the Bau protein. Numerous types of appropriate expression vectors are known in the art for mammalian (including human) protein expression, by standard molecular biology techniques. Such vectors may
5 be selected from among conventional vector types including insects, e.g., baculovirus expression, or yeast, fungal, bacterial or viral expression systems. Other appropriate expression vectors, of which numerous types are known in the art, can also be used for this
10 purpose.

Methods for obtaining such expression vectors are well-known. See, Sambrook et al, Molecular Cloning. A Laboratory Manual, 2d edition, Cold Spring Harbor Laboratory, New York (1989); Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references
15 cited therein.

Suitable host cells or cell lines for transfection by this method include mammalian cells, such as Human 293 cells, Chinese hamster ovary cells (CHO),
20 the monkey COS-1 cell line or murine 3T3 cells derived from Swiss, Balb-c or NIH mice may be used. Another suitable mammalian cell line is the CV-1 cell line. Still other suitable mammalian host cells, as well as methods for transfection, culture, amplification,
25 screening, production, and purification are known in the art. [See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U. S. Patent 4,419,446].

30 Similarly bacterial cells are useful as host cells for the present invention. For example, the various strains of *E. coli* (e.g., HB101, MC1061, and strains used in the following examples) are well-known as host cells in the field of biotechnology. Various

strains of *B. subtilis*, *Pseudomonas*, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Other fungal cells may also be employed as expression systems. Alternatively, insect cells such as *Spodoptera frugiperda* (Sf9) cells may be used.

Thus, the present invention provides a method for producing a recombinant Bau protein which involves transfecting a host cell with at least one expression vector containing a recombinant polynucleotide encoding a Bau protein under the control of a transcriptional regulatory sequence, e.g., by conventional means such as electroporation. The transfected host cell is then cultured under conditions that allow expression of the Bau protein. The expressed protein is then recovered, isolated, and optionally purified from the culture medium (or from the cell, if expressed intracellularly) by appropriate means known to one of skill in the art.

For example, the proteins may be isolated in soluble form following cell lysis, or may be extracted using known techniques, e.g., in guanidine chloride. If desired, the Bau proteins of the invention may be produced as a fusion protein. For example, it may be desirable to produce Bau fusion proteins, to enhance expression of the protein in a selected host cell, to improve purification, or for use in monitoring the presence of Bau in tissues, cells or cell extracts. Suitable fusion partners for the Bau proteins of the invention are well known to those of skill in the art and include, among others, β -galactosidase, glutathione-S-transferase, and poly-histidine.

B. In Vivo

Alternatively, where it is desired that the Bau protein be expressed *in vivo*, e.g., for gene therapy purposes, an appropriate vector for delivery of Bau, or
5 fragment thereof, may be readily selected by one of skill in the art. Exemplary gene therapy vectors are readily available from a variety of academic and commercial sources, and include, e.g., adeno-associated virus [International patent application No. PCT/US91/03440],
10 adenovirus vectors [M. Kay et al, Proc. Natl. Acad. Sci. USA, 91:2353 (1994); S. Ishibashi et al, J. Clin. Invest., 92:883 (1993)], or other viral vectors, e.g., various poxviruses, vaccinia, etc. Methods for insertion of a desired gene, e.g. Bau, and obtaining *in vivo*
15 expression of the encoded protein, are well known to those of skill in the art.

IV. Antisera and Antibodies

The Bau proteins of this invention are also useful as antigens for the development of anti-Bau antisera and
20 antibodies to Bau or to a desired fragment of a Bau protein. Specific antisera may be generated using known techniques. See, Sambrook, cited above, Chapter 18, generally, incorporated by reference. Similarly, antibodies of the invention, both polyclonal and
25 monoclonal, may be produced by conventional methods, including the Kohler and Milstein hybridoma technique, recombinant techniques, such as described by Huse et al, Science, 246:1275-1281 (1988), or any other techniques known to the art.

30 Also encompassed within this invention are humanized and chimeric antibodies. As used herein, a humanized antibody is defined as an antibody containing murine complementary determining regions (CDRs) capable of binding to Bau or a fragment thereof, and human framework

regions. These CDRs are preferably derived from a murine monoclonal antibody (MAb) of the invention. As defined herein, a chimeric antibody is defined as an antibody containing the variable region light and heavy chains, including both CDR and framework regions, from a Bau MAb of the invention and the constant region light and heavy chains from a human antibody. Methods of identifying suitable human framework regions and modifying a MAb of the invention to contain same to produce a humanized or chimeric antibody of the invention, are well known to those of skill in the art. See, e.g., E. Mark and Padlin, "Humanization of Monoclonal Antibodies", Chapter 4, The Handbook of Experimental Pharmacology, Vol. 113, The Pharmacology of Monoclonal Antibodies, Springer-Verlag (June 1994). Other types of recombinantly-designed antibodies are also encompassed by this invention.

Further provided by the present invention are anti-idiotypic antibodies (Ab2) and anti-anti-idiotypic antibodies (Ab3). Ab2 are specific for the target to which anti-Bau antibodies of the invention bind and Ab3 are similar to Bau antibodies (Ab1) in their binding specificities and biological activities [see, e.g., M. Wettendorff et al, "Modulation of anti-tumor immunity by anti-idiotypic antibodies." In Idiotypic Network and Diseases, ed. by J. Cerny and J. Hiernaux J, Am. Soc. Microbiol., Washington DC: pp. 203-229, (1990)]. These anti-idiotypic and anti-anti-idiotypic antibodies may be produced using techniques well known to those of skill in the art. Such anti-idiotypic antibodies (Ab2) can bear the internal image of Bau and can thus bind to Bin1 in much the same manner as Bau, and are thus useful for the same purposes as Bau.

In general, polyclonal antisera, monoclonal antibodies and other antibodies which bind to Bau as the

antigen (Ab1) are useful to identify epitopes of Bau, to separate Bau from contaminants in living tissue (e.g., in chromatographic columns and the like), and in general as research tools and as starting material essential for the development of other types of antibodies described above. Anti-idiotypic antibodies (Ab2) are useful for binding BIN1 and thus may be used in the treatment of cancers in which BIN1 can affect c-MYC, which is part of a biochemical cascade of events leading to tumor formation. The Ab3 antibodies may be useful for the same reason the Ab1 are useful. Other uses as research tools and as components for separation of Bin from other contaminants of living tissue, for example, are also contemplated for these antibodies.

V. Diagnostic Reagents and Methods

Advantageously, the present invention provides reagents and methods useful in detecting and diagnosing abnormal levels of Bau, and particularly deficiencies, mutant species, or excess production of either, in a patient. As defined herein, a deficiency of Bau is an inadequate amount of Bau to compensate for the levels of BIN1 and c-MYC in a patient. Conditions associated with deficiencies of Bau may include a variety of cancers, e.g., breast cancer, liver cancer and colon cancer, and hyperplastic disease states, e.g., benign prostate hyperplasia, involving MYC activation.

Thus, the proteins, protein fragments, antibodies, and polynucleotide sequences (including anti-sense polynucleotide sequences and oligonucleotide fragments), and Bau antisera and antibodies of this invention may be useful as diagnostic reagents. These reagents may optionally be labelled using diagnostic labels, such as radioactive labels, colorimetric enzyme label systems and the like conventionally used in diagnostic or therapeutic

methods. Alternatively, the N- or C-terminus of Bau or a fragment thereof may be tagged with a viral epitope which can be recognized by a specific antisera. The reagents may be used to measure abnormal Bau levels in selected mammalian tissue in conventional diagnostic assays, e.g., Southern blotting, Northern and Western blotting, polymerase chain reaction (PCR), reverse transcriptase (RT) PCR, immunostaining, and the like. For example, in biopsies of tumor tissue, loss of Bau expression in tumor tissue could be directly verified by RT-PCR or immunostaining. Alternatively, a Southern analysis, genomic PCR, or fluorescence *in situ* hybridization (FISH) may be performed to confirm Bau1 gene rearrangement.

In one example, as diagnostic agents the polynucleotide sequences may be employed to detect or quantitate normal Bau. The selection of the appropriate assay format and label system is within the skill of the art and may readily be chosen without requiring additional explanation by resort to the wealth of art in the diagnostic area.

Thus the present invention provides methods for the detection of disorders characterized by insufficient Bau levels. The methods involve contacting a selected mammalian tissue, e.g., a biopsy sample or other cells, with the selected reagent, protein, antisera antibody or DNA sequence, and measuring or detecting the amount of Bau present in the tissue in a selected assay format based on the binding or hybridization of the reagent to the tissue.

VI. Therapeutic Compositions and Methods

Compositions and methods useful for the treatment of conditions associated with inadequate Bau levels are provided. As stated above, included among such conditions are cancers involving MYC activation. Also

provided are compositions and methods for inhibiting Bau activity in order to ameliorate a condition in which apoptosis is activated and BIN1 plays a role. Such conditions may include degenerative conditions, e.g.,
5 neurodegenerative diseases.

For example, where it is desirable to augment Bau activity in order to increase Bin1-mediated tumor suppressor activity or decrease the malignant activity of deregulated Myc, a therapeutic composition of the
10 invention may be prepared which contains a Bau polypeptide, or a peptidomimetic drug derived from a Bau using the techniques described herein. Other Bau agonists, e.g., those identified using the methods described in Section VII below, are suitable components
15 for a therapeutic composition which inhibits Bau activity. Such antagonists may be used in conjunction with Bau polypeptides or as alternatives thereto.

In other circumstances, such as degenerative diseases, it may be desirable to prepare therapeutic
20 compositions which contain a Bau antagonist, such as a Bau antibody. One particularly desirable antibody would be directed against the Bin1-binding domain of Bau, located within aa 62 to 209 of SEQ ID NO:2. Other Bau antagonists, e.g., those identified using the methods
25 described in Section VII below, may also be useful. Such antagonists may be used in conjunction with anti-Bau antibodies, or as alternatives thereto.

The therapeutic composition of the invention desirably contains 0.01 μ g to 10 mg protein. These
30 compositions may contain a pharmaceutically acceptable carrier. Suitable carriers are well known to those of skill in the art and include, for example, saline. Alternatively, such compositions may include conventional delivery systems into which protein of the invention is

incorporated. Optionally, these compositions may contain other active ingredients, e.g., chemotherapeutics.

Still another method involves the use of the Bau polynucleotide sequences for gene therapy. In the method, the Bau sequences are introduced into a suitable vector for delivery to a cell containing a deficiency of Bau and/or BIN1 levels. By conventional genetic engineering techniques, the Bau gene sequence may be introduced to mutate the existing gene by recombination or to replace an inactive or missing gene.

Generally, a suitable polynucleotide-based treatment contains between 1×10^{-3} pfu to 1×10^{12} pfu per dose. However, the dose, timing and mode of administration of these compositions may be determined by one of skill in the art. Such factors as the age, condition, and the level of the Bau deficiency detected by the diagnostic methods described above, may be taken into account in determining the dose, timing and mode of administration of the therapeutic compositions of the invention. Generally, where treatment of an existing cancer or hyperplastic state is indicated, a therapeutic composition of the invention is preferably administered in a site-directed manner and is repeated as needed. Such therapy may be administered in conjunction with conventional therapies, including radiation and/or chemotherapeutic treatments.

VII. Drug Screening and Development

The proteins, antibodies and polynucleotide sequences of the present invention may also be used in the screening and development of chemical compounds or proteins which have utility as therapeutic drugs for the treatment of cancers characterized by Bau or BIN1, which regulate inappropriate MYC levels. As one example, a compound capable of binding to Bau and preventing its

biological activity may be a useful drug component for the treatment or prevention of cancer. The methods described herein may also be applied to fragments of Bau. One particularly suitable fragment is the Bin1-binding domain (aa 62 to 209 of SEQ ID NO:2).

Suitable assay methods may be readily determined by one of skill in the art. Where desired, and depending on the assay selected, Bau may be immobilized directly or indirectly (e.g., via an anti-Bau antibody) on a suitable surface, e.g., in an ELISA format. Such immobilization surfaces are well known. For example, a wettable inert bead may be used. Alternatively, Bau may be used in screening assays which do not require immobilization, e.g., in the screening of combinatorial libraries.

Assays and techniques exist for the screening and development of drugs capable of binding to selected regions of Bau. These include the use of phage display system for expressing the Bau proteins, and using a culture of transfected *E. coli* or other microorganism to produce the proteins for binding studies of potential binding compounds. See, for example, the techniques described in G. Cesarini, FEBS Letters, 307(1):66-70 (July 1992); H. Gram et al., J. Immunol. Meth., 161:169-176 (1993); C. Summer et al., Proc. Natl. Acad. Sci. USA, 89:3756-3760 (May 1992), incorporated by reference herein.

Other conventional drug screening techniques may be employed using the proteins, antibodies or polynucleotide sequences of this invention. As one example, a method for identifying compounds which specifically bind to a Bau protein can include simply the steps of contacting a selected Bau protein with a test compound to permit binding of the test compound to Bau; and determining the amount of test compound, if any, which is bound to the

Bau protein. Such a method may involve the incubation of the test compound and the Bau protein immobilized on a solid support.

Typically, the surface containing the immobilized
5 ligand is permitted to come into contact with a solution containing the Bau protein and binding is measured using an appropriate detection system. Suitable detection systems include the streptavidin horse radish peroxidase conjugate, direct conjugation by a tag, e.g.,
10 fluorescein. Other systems are well known to those of skill in the art. This invention is not limited by the detection system used.

Another method of identifying compounds which specifically bind to Bau can include the steps of
15 contacting a Bau protein immobilized on a solid support with both a test compound and the protein sequence which is a receptor for Bau to permit binding of the receptor to the Bau protein; and determining the amount of the receptor which is bound to the Bau protein. The
20 inhibition of binding of the normal protein by the test compound thereby indicates binding of the test compound to the Bau protein.

Thus, through use of such methods, the present invention is anticipated to provide compounds capable of
25 interacting with Bau or portions thereof, and either enhancing or decreasing its biological activity, as desired. Such compounds are believed to be encompassed by this invention.

The assay methods described herein are also useful
30 in screening for inhibition of the interaction between a Bau protein of the invention and BIN1 and/or another ligand(s). The solution containing the inhibitors may be obtained from any appropriate source, including, for example, extracts of supernatants from culture of

bioorganisms, extracts from organisms collected from natural sources, chemical compounds, and mixtures thereof.

The following examples illustrate the isolation and use of the Bau nucleic acid sequences, polypeptides, and fragments of the invention. These examples are illustrative only and do not limit the scope of the invention.

Example 1 - Identification and Characterization of Bau

A yeast two hybrid screen was performed to identify U1-specific BIN1-binding proteins.

The two hybrid system and methodology used for the screen was similar to that used to identify the MYC-interacting protein BIN1 [D. Sakamuro, et al, cited above]. A region of BIN1 (aa 214-269 of SEQ ID NO:6) that included exon 9 (U1) and 10 sequences was used as the "bait" polypeptide. U1-binding clones were screened from a murine 10.5d embryo cDNA library [Vojtek et al, Cell, 74: 205-214 (1993)] on the basis of their ability to confer HIS⁺ and LacZ⁺ phenotypes to the yeast assay strain L40, after transformation with "bait" and cDNA library plasmids. A mating strategy [Vojtek et al, Cell, 74:205-214 (1993)] was used to identify the U1-specific clones, using the control "baits" lamin, rhoB [Sakamuro et al, cited above], and a deletion mutant of the U1 region (containing only aa 214-229). cDNA library plasmids from U1-specific clones were shuttled into *E. coli* and subjected to DNA sequencing.

By this approach, two different sequences encoding U1-binding polypeptides were identified. One of the sequence classes encoded an N-terminal region of nucleophosmin, a growth-regulated protein which shuttles between the nucleolus and the nucleoplasm and is believed to play a role in the regulation of ribosome

biosynthesis [Yung and Chou, Biochem. Biophys. Res. Comm., 217:313-325 (1995)]. Interestingly, oncogenic translocations of nucleophosmin occur in certain non-Hodgkin's lymphomas, and Myc is known to regulate
5 ribosomal RNA synthesis.

The second sequence was unrelated to other sequences in the DNA database (data not shown). A ~1 kb near-full length cDNA was obtained from a murine embryonic phage library by standard hybridization methodology and its
10 entire DNA sequence was determined (SEQ ID NO: 1). The DNA sequence of this clone, termed Bau (for Bin1-Associated U1-specific protein), encoded a 293 amino acid hydrophilic polypeptide of predicted molecular weight 34,137 Daltons (SEQ ID NO: 2). Structure prediction
15 analysis of Bau using the COILS algorithm [A. Lupas, Meth Enz. 266: 513-525 (1996)] indicated that its N-terminal region (aa 1-160) is strongly predicted to form a coiled-coil domain (data not shown).

Example 2 - Bau contains motifs found in cysteine
20 protease inhibitors and a mitotic checkpoint regulator

Comparison of Bau sequences to the DNA database revealed no significant relationship to known gene products. However, analysis of Bau sequence using the BLOCKS algorithm [Henikoff and Henikoff, Genomics, 19:
25 97-107 (1994)], which identifies signature motifs, turned up a region shared with type I cystatins, a class of cysteine protease inhibitors. (Signature motifs are sequence homology motifs that are found in all members of a polypeptide family with similar functional
30 characteristics.) Type I cystatins are ~100 aa molecules that form non-disulfide linked dimers. The signature motif in Bau is located at aa 89-105 and was 65% (11/17) identical and 76% (13/17) similar to cystatin B [A. Machleidt et al., Biochem Biophys Res Commun, 131: 1187-

1192 (1985)], a liver thiol protease inhibitor of the cystatin type I family most closely related to Bau.

The cystatin similarity suggests that Bau may be able to inhibit certain cysteine protease inhibitors.

5 This is significant because (i.) a class of ubiquitous cysteine protease inhibitors termed caspases have a effector central role in activating apoptosis, and (ii.) Bin1 has been implicated in mediating apoptosis by Myc. Based on this relationship, Bau is hypothesized to
10 function in a Bin1-Bau-caspase signaling pathway for apoptosis.

By visual inspection, an additional relationship was identified between the Bau-cystatin motif and the yeast checkpoint regulator Mad1p [Hardwick and Murray, J Cell Biol, 131: 709-720 (1995)]. Mad1p arrests mitosis in
15 response to spindle assembly damage. Mad1p was one of a set of mitotic regulators that was examined for a suspected relationship to Bau, because of existing links between Bin1 and mitosis, namely, a localization of Bin1
20 at the microtubule organizing center (MTOC; spindle pole) during mitosis and an MTOC-like structure in cells destined to undergo apoptosis (G.P., unpublished observations). While it is not known yet if Bau has a mitotic role, or if Mad1p can inhibit cysteine protease
25 activity, the Bau-cystatin motif identified in Mad1p is located at aa 83-103 and exhibits 57% (12/21) identity and 71% (15/21) similarity to the others. Bau and Mad1p also share similarity consisting of a polyasparagine region, at aa 165-169 and aa 330-372, respectively,
30 downstream of the Bau-cystatin motif. Consistent with a functional link to apoptosis, spindle pole damage following loss of Mad1p results in an apoptosis-like death in yeast. Taken together, the structural

relationship between Bau, type I cystatins, and Mad1p are consistent with a role for Bau in the regulation of Bin1-mediated apoptosis.

5 Example 3 - Bin1 exons 9 (U1) and 10 are necessary for Bau interaction

 The original two hybrid cDNAs encoded an open reading frame of at approximately 145 amino acids. This region, which constitutes a Bin1-binding domain (BBD) sufficient for Bin1 interaction, is located at aa 62-209
10 of Bau [SEQ ID NO:2].

 To confirm the interaction between Bau and Bin1, and to define the exact regions of Bin1 required for interaction with Bau, the following *in vitro* biochemical analysis was performed. Various regions of Bin1,
15 including U1, the adjacent NLS-like motif (exon 10 sequences), or U1-NLS, were expressed in *E. coli* by fusing them to glutathione-S-transferase (GST), using standard methodology. Unfused GST or GST fusions were purified by glutathione affinity purification, using
20 standard methods, and employed for Bau binding experiments. Bau was engineered with a epitope tag and translation termination site at its 3' end; the Bau BBD was engineered for expression with a Kozak translation initiation site (the two hybrid cDNA subcloned included a
25 termination site at its 3' end). For binding assays, equivalent amounts of GST proteins were mixed with ³⁵S-methionine labeled Bau or BBD, expressed by *in vitro* translation in rabbit reticulocyte extracts.

 Both Bau and the BBD were each observed to bind
30 specifically to GST-U1-NLS but more poorly or not at all to GST-U1 or GST-NLS. The interaction of Bau was slightly weaker than BBD, which stable at near physiological salt concentrations (150 mM NaCl) and in 0.1% NP40 (a nonionic detergent). In addition, under low

salt conditions (50 mM NaCl), BBD could also bind weakly to GST-NLS, a region insufficient for Bau binding under any conditions. These data were confirmed for BBD in the two hybrid system.

5 The results indicated that Bau association depended upon both U1 and the NLS-like motif encoded by exon 10, which was spliced into Bin1 message following induction of either cell differentiation (in the absence of Myc) or apoptosis (in the presence of deregulated Myc). These
10 findings supported the hypothesis that Bau may participate in mediating or regulating Bin1 function during such cell fate changes.

Example 4 - Bau is encoded by a single copy,
evolutionarily conserved gene that is widely expressed

15 To obtain evidence that Bau was a novel gene product, Southern and Northern analyses of genomic DNA and cytoplasmic RNA from murine and human cells were performed, using standard methods. Southern blots hybridized with the murine Bau cDNA revealed the presence
20 of a single-copy bands in both murine and human DNA. The presence of evolutionarily conserved sequences strongly argued for gene identification. On Northern blots of RNA isolated from embryonic and adult murine tissues and from various human cell lines, a ubiquitously expressed RNA of
25 approximately 1.4 kb was detected. In certain human tumor cells, such as HepG2 hepatocarcinoma cells, Bau message was not detected. Since normal murine liver expressed Bau, the lack of message in HepG2 cells suggested that Bau expression may be lost during the
30 genesis of certain types of cancer such as hepatocarcinoma. Taken together, the data supported the assertion that Bau is encoded by a novel gene which has a tumor suppressor role like Bin1.

Example 5 - Bau inhibits malignant cell transformation

Bin1 can inhibit malignant cell transformation by Myc but also by the adenovirus E1A oncoprotein, through a Myc-independent mechanism [D. Sakamuro, et al., Nature Genet, 14: 69-77 (1996)]. Inhibition of Myc is partially dependent and E1A completely dependent upon the integrity of the U1 region in Bin1 [Elliott, Sakamuro et al., manuscript submitted]. Since Bau can interact with U1, we speculated that Bau might also inhibit cell transformation by Myc or E1A. To test this, Bau was assayed for the ability to suppress transformed focus formation in rat embryo fibroblasts (REFs) induced by these oncoproteins. Bau was subcloned into a mammalian expression vector so it could be tested in this assay. In addition, to assay any effect of the BBD, it was similarly subcloned for testing. As a negative control for any effects on transformation, we tested the effects of Bau or BBD on SV40 T antigen, which but is unaffected by Bin1 in this assay [D. Sakamuro, et al., cited above]. Bau and BBD was observed to inhibit the activity of E1A in this assay approximately 3-fold and 4-fold, respectively (see Figure 1). While BBD suppressed the activity of Myc approximately 2-fold, Bau had a lesser effect that was not statistically significant (data not shown). This effect may reflect the lesser dependence of Myc for U1 in this assay. The inhibition of E1A was specific insofar as neither Bau nor BBD significantly affected transformation by T antigen. Consistent with a lack of inhibition in T antigen-transformed cells, exogenous message was detected in RNA isolated from pools of transformed colonies by Northern analysis. Taken together, the results supported the conclusion that Bau was a growth inhibitor that could interfere with certain types of malignant cell proliferation.

Example 6 - Isolation of Human Bau cDNA

Using the murine cDNA as a probe, the human Bau cDNA can be obtained from a human HeLa cell λ ZAPII cDNA library (Stratagene, La Jolla, CA) by standard methods [Sambrook et al, cited above], i.e., by hybridization with [32 P]-labeled Bau and washing under low stringency conditions (2 x SSC 42°C). The complete sequence of the human cDNA can be determined using the dideoxy method with Sequenase (US Biochemicals) and assembled and analyzed with MacVector software (IBI/Kodak).

Example 7 - Anti-Bau Antibodies

For use in generating antibodies, the Bau sequences encoding the Bin1-binding domain (BBD, aa 62 to 209 of SEQ ID NO:2) was expressed as a glutathione-S-transferase (GST) fusion protein.

To construct the GST fusion protein, the partial Bau cDNA initially isolated in the two hybrid screen was used (this cDNA encoded only the BBD as defined above). A Bam HI-Eco RI fragment including the partial cDNA was isolated from the two hybrid vector and shuttled into the baculovirus recombination vector pAcGHLT-C (Invitrogen, Inc., San Diego, CA). The recombinant plasmid was introduced into Sf9 insect cells. The GST-Bau₆₂₋₂₀₉ polypeptide whose synthesis was directed by the recombinant plasmid was purified from Sf9 cell extracts on glutathione-Sepharose (Pharmacia), using protocols supplied by the vendor.

To generate BBD-specific antibodies, GST-Bau₆₂₋₂₀₉ was used to immunize mice. Cells prepared from the spleens of immunized mice were processed for hybridoma production and monoclonal antibody purification, using standard protocols.

All documents cited above are incorporated herein by reference. Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of
5 skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: The Wistar Institute of Anatomy and Biology
- (ii) TITLE OF INVENTION: Bau, A Binl Interacting Protein, and Uses Therefor
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Howson and Howson
 - (B) STREET: Spring House Corporate Cntr, P.O. Box 457
 - (C) CITY: Spring House
 - (D) STATE: Pennsylvania
 - (E) COUNTRY: USA
 - (F) ZIP: 19477
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/025,482
 - (B) FILING DATE: 29-AUG-1996
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/
 - (B) FILING DATE: 27-AUG-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Kodroff, Cathy A.
 - (B) REGISTRATION NUMBER: 33,980
 - (C) REFERENCE/DOCKET NUMBER: WST73APCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 215-540-9200
 - (B) TELEFAX: 215-540-5818

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 926 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 48..926

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | |
|---|-------------|-----|
| GGGGTGACGT GGCCATTGAG GTCTTTGAGC TGCCTGAGAA CGAGGAC | ATG TTT TCC | 56 |
| | Met Phe Ser | |
| | 1 | |
| CCA TCT GAC CTG GAC ACA AGC AAG CTC AGC CAC AAG TTC AAA GAG TTG | | 104 |
| Pro Ser Asp Leu Asp Thr Ser Lys Leu Ser His Lys Phe Lys Glu Leu | | |
| 5 10 15 | | |
| CAA ATC AAA CAT GCA GTT ACA GAA GCA GAG ATT CAA AAA TTG AAG ACC | | 152 |
| Gln Ile Lys His Ala Val Thr Glu Ala Glu Ile Gln Lys Leu Lys Thr | | |
| 20 25 30 35 | | |
| AAG CTT CAA GCA TCC GAA AAT GAG AAA GTA AGG TGG GAA CTA GAA AAG | | 200 |
| Lys Leu Gln Ala Ser Glu Asn Glu Lys Val Arg Trp Glu Leu Glu Lys | | |
| 40 45 50 | | |
| AAC CAA CTG CAA CAG AAT ATA GAA GAG AAT AAA GAA CGG ATG CTG AAG | | 248 |
| Asn Gln Leu Gln Gln Asn Ile Glu Glu Asn Lys Glu Arg Met Leu Lys | | |
| 55 60 65 | | |
| TTG GAG AGC TAC TGG ATC GAG GCT CAG ACA TTA TGT CAT ACG GTG AAT | | 296 |
| Leu Glu Ser Tyr Trp Ile Glu Ala Gln Thr Leu Cys His Thr Val Asn | | |
| 70 75 80 | | |
| GAG CAT CTC AAA GAG ACT CAG AGC CAG TAC CAA GCC CTG GAA AAG AAA | | 344 |
| Glu His Leu Lys Glu Thr Gln Ser Gln Tyr Gln Ala Leu Glu Lys Lys | | |
| 85 90 95 | | |
| TAC AAC AAA GCA AAG AAG CTG ATC AAA GAC TTC CAG CAA AAA GAG CTC | | 392 |
| Tyr Asn Lys Ala Lys Lys Leu Ile Lys Asp Phe Gln Gln Lys Glu Leu | | |
| 100 105 110 115 | | |
| GAT TTC ATC AAG AGA CAG GAA GTA GAA AGA AAG AAG CGG GAG GAG GTG | | 440 |
| Asp Phe Ile Lys Arg Gln Glu Val Glu Arg Lys Lys Arg Glu Glu Val | | |
| 120 125 130 | | |
| GAA AAG GCT CAC CTG CTT GAA GTC CAA GGC CTG CAA GTT CGG ATT AGA | | 488 |
| Glu Lys Ala His Leu Leu Glu Val Gln Gly Leu Gln Val Arg Ile Arg | | |
| 135 140 145 | | |
| GAT TTG GAG GCT GAG GTG TTC AGA CTA CTA AAG CAA AAT GGG ACC CAG | | 536 |
| Asp Leu Glu Ala Glu Val Phe Arg Leu Leu Lys Gln Asn Gly Thr Gln | | |
| 150 155 160 | | |
| GTT AAC AAC AAC AAC AAC ATC TTT GAG AGA AGA CCA TCT CCC GGG GAA | | 584 |
| Val Asn Asn Asn Asn Ile Phe Glu Arg Arg Pro Ser Pro Gly Glu | | |
| 165 170 175 | | |
| GTC TCG AAA GGA GAC ACT ATG GAG AAT GTG GAA GTC AAG CAA ACA TCC | | 632 |
| Val Ser Lys Gly Asp Thr Met Glu Asn Val Glu Val Lys Gln Thr Ser | | |
| 180 185 190 195 | | |
| TGT CAG GAC GGC TTG AGC CAA GAC CTG AAT GAA GCA GTC CCA GAG ACA | | 680 |
| Cys Gln Asp Gly Leu Ser Gln Asp Leu Asn Glu Ala Val Pro Glu Thr | | |
| 200 205 210 | | |
| GAG CGC CTG GAT TCG AAA GCA TTG AAA ACC CGG GCC CAG CTC TCT GTG | | 728 |
| Glu Arg Leu Asp Ser Lys Ala Leu Lys Thr Arg Ala Gln Leu Ser Val | | |
| 215 220 225 | | |

31

| | |
|---|-----|
| AAG AAC AGG CGC CAG AGG CCC ACA AGG ACA CGG CTC TAT GAC AGC GTC Lys Asn Arg Arg Gln Arg Pro Thr Arg Thr Arg Leu Tyr Asp Ser Val 230 235 240 | 776 |
| AGC TCA ACT GAT GGG GAG GAC AGC CTG GAG AGG AAG GTG AGC ACT CTC Ser Ser Thr Asp Gly Glu Asp Ser Leu Glu Arg Lys Val Ser Thr Leu 245 250 255 | 824 |
| AAT GGC TGG CAG ACT CTT GCA GAG TGT CGT TGT CCA CCA GTG TAT TTA Asn Gly Trp Gln Thr Leu Ala Glu Cys Arg Cys Pro Pro Val Tyr Leu 260 265 270 275 | 872 |
| TTG AAC GTG ATA GCG GTT TTA CTG ATC TGT GCC TTA CTT GGA AGA AAG Leu Asn Val Ile Ala Val Leu Leu Ile Cys Ala Leu Leu Gly Arg Lys 280 285 290 | 920 |
| TCT CCC Ser Pro | 926 |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 293 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| |
|--|
| Met Phe Ser Pro Ser Asp Leu Asp Thr Ser Lys Leu Ser His Lys Phe 1 5 10 15 |
| Lys Glu Leu Gln Ile Lys His Ala Val Thr Glu Ala Glu Ile Gln Lys 20 25 30 |
| Leu Lys Thr Lys Leu Gln Ala Ser Glu Asn Glu Lys Val Arg Trp Glu 35 40 45 |
| Leu Glu Lys Asn Gln Leu Gln Gln Asn Ile Glu Glu Asn Lys Glu Arg 50 55 60 |
| Met Leu Lys Leu Glu Ser Tyr Trp Ile Glu Ala Gln Thr Leu Cys His 65 70 75 80 |
| Thr Val Asn Glu His Leu Lys Glu Thr Gln Ser Gln Tyr Gln Ala Leu 85 90 95 |
| Glu Lys Lys Tyr Asn Lys Ala Lys Lys Leu Ile Lys Asp Phe Gln Gln 100 105 110 |
| Lys Glu Leu Asp Phe Ile Lys Arg Gln Glu Val Glu Arg Lys Lys Arg 115 120 125 |
| Glu Glu Val Glu Lys Ala His Leu Leu Glu Val Gln Gly Leu Gln Val 130 135 140 |
| Arg Ile Arg Asp Leu Glu Ala Glu Val Phe Arg Leu Leu Lys Gln Asn 145 150 155 160 |

32

Gly Thr Gln Val Asn Asn Asn Asn Asn Ile Phe Glu Arg Arg Pro Ser
 165 170 175
 Pro Gly Glu Val Ser Lys Gly Asp Thr Met Glu Asn Val Glu Val Lys
 180 185 190
 Gln Thr Ser Cys Gln Asp Gly Leu Ser Gln Asp Leu Asn Glu Ala Val
 195 200 205
 Pro Glu Thr Glu Arg Leu Asp Ser Lys Ala Leu Lys Thr Arg Ala Gln
 210 215 220
 Leu Ser Val Lys Asn Arg Arg Gln Arg Pro Thr Arg Thr Arg Leu Tyr
 225 230 235 240
 Asp Ser Val Ser Ser Thr Asp Gly Glu Asp Ser Leu Glu Arg Lys Val
 245 250 255
 Ser Thr Leu Asn Gly Trp Gln Thr Leu Ala Glu Cys Arg Cys Pro Pro
 260 265 270
 Val Tyr Leu Leu Asn Val Ile Ala Val Leu Leu Ile Cys Ala Leu Leu
 275 280 285
 Gly Arg Lys Ser Pro
 290

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..399

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | |
|---|-----|
| GAG ATC AGA GTG AAC CAT GAG CCA GAG CCG GCC AGT GGG GCC TCA CCC | 48 |
| Glu Ile Arg Val Asn His Glu Pro Glu Pro Ala Ser Gly Ala Ser Pro | |
| 1 5 10 15 | |
| GGG GCT GCC ATC CCC AAG TCC CCA TCT CAG CCA GCA GAG GCC TCC GAG | 96 |
| Gly Ala Ala Ile Pro Lys Ser Pro Ser Gln Pro Ala Glu Ala Ser Glu | |
| 20 25 30 | |
| GTG GTG GGT GGA GCC CAG GAG CCA GGG GAG ACA GCA GCC AGT GAA GCA | 144 |
| Val Val Gly Gly Ala Gln Glu Pro Gly Glu Thr Ala Ala Ser Glu Ala | |
| 35 40 45 | |
| ACC TCC AGC TCT CTT CCG GCT GTG GTG GTG GAG ACC TTC TCC GCA ACT | 192 |
| Thr Ser Ser Ser Leu Pro Ala Val Val Val Glu Thr Phe Ser Ala Thr | |
| 50 55 60 | |
| GTG AAT GGG GCG GTG GAG GGC AGC GCT GCG ACT GGA CGC TTG GAC CTG | 240 |
| Val Asn Gly Ala Val Glu Gly Ser Ala Gly Thr Gly Arg Leu Asp Leu | |
| 65 70 75 80 | |

33

| | |
|---|-----|
| CCC CCG GGA TTC ATG TTC AAG GTT CAA GCC CAG CAT GAT TAC ACG GCC | 288 |
| Pro Pro Gly Phe Met Phe Lys Val Gln Ala Gln His Asp Tyr Thr Ala | |
| 85 90 95 | |
| ACT GAC ACT GAT GAG CTG CAA CTC AAA GCT GGC GAT GTG GTG TTG GTG | 336 |
| Thr Asp Thr Asp Glu Leu Gln Leu Lys Ala Gly Asp Val Val Leu Val | |
| 100 105 110 | |
| ATT CCT TTC CAG AAC CCA GAG GAG CAG GAT GAA GGC TGG CTC ATG GGT | 384 |
| Ile Pro Phe Gln Asn Pro Glu Glu Gln Asp Glu Gly Trp Leu Met Gly | |
| 115 120 125 | |
| GTG AAG GAG AGC GAC TGA | 402 |
| Val Lys Glu Ser Asp | |
| 130 | |

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 133 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | |
|---|--|
| Glu Ile Arg Val Asn His Glu Pro Glu Pro Ala Ser Gly Ala Ser Pro | |
| 1 5 10 15 | |
| Gly Ala Ala Ile Pro Lys Ser Pro Ser Gln Pro Ala Glu Ala Ser Glu | |
| 20 25 30 | |
| Val Val Gly Gly Ala Gln Glu Pro Gly Glu Thr Ala Ala Ser Glu Ala | |
| 35 40 45 | |
| Thr Ser Ser Ser Leu Pro Ala Val Val Val Glu Thr Phe Ser Ala Thr | |
| 50 55 60 | |
| Val Asn Gly Ala Val Glu Gly Ser Ala Gly Thr Gly Arg Leu Asp Leu | |
| 65 70 75 80 | |
| Pro Pro Gly Phe Met Phe Lys Val Gln Ala Gln His Asp Tyr Thr Ala | |
| 85 90 95 | |
| Thr Asp Thr Asp Glu Leu Gln Leu Lys Ala Gly Asp Val Val Leu Val | |
| 100 105 110 | |
| Ile Pro Phe Gln Asn Pro Glu Glu Gln Asp Glu Gly Trp Leu Met Gly | |
| 115 120 125 | |
| Val Lys Glu Ser Asp | |
| 130 | |

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1925 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

34

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 60..1412

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | | | | | | | | | | | | | | | | |
|---------|-----|---------|-----|----------|-----|---------|-----|----------|-----|---------|-----|-----|-----|-----|-----|-----|
| GAATTCG | GTG | CTGGTTG | GAG | TTGCTCAT | CT | CCTTGTG | GAA | GTTTTCCT | CC | AGGCCCG | CG | | 59 | | | |
| ATG | CTC | TGG | AAC | GTG | GTG | ACG | GCG | GGA | AAG | ATC | GCC | AGC | AAC | GTG | CAG | 107 |
| Met | Leu | Trp | Asn | Val | Val | Thr | Ala | Gly | Lys | Ile | Ala | Ser | Asn | Val | Gln | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| AAG | AAG | CTC | ACC | CGC | GCG | CAG | GAG | AAG | GTT | CTC | CAG | AAG | CTG | GGG | AAG | 155 |
| Lys | Lys | Leu | Thr | Arg | Ala | Gln | Glu | Lys | Val | Leu | Gln | Lys | Leu | Gly | Lys | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| GCA | GAT | GAG | ACC | AAG | GAT | GAG | CAG | TTT | GAG | CAG | TGC | GTC | CAG | AAT | TTC | 203 |
| Ala | Asp | Glu | Thr | Lys | Asp | Glu | Gln | Phe | Glu | Gln | Cys | Val | Gln | Asn | Phe | |
| | | 35 | | | | | 40 | | | | | 45 | | | | |
| AAC | AAG | CAG | CTG | ACG | GAG | GGC | ACC | CGG | CTG | CAG | AAG | GAT | CTC | CGG | ACC | 251 |
| Asn | Lys | Gln | Leu | Thr | Glu | Gly | Thr | Arg | Leu | Gln | Lys | Asp | Leu | Arg | Thr | |
| | 50 | | | | | 55 | | | | | 60 | | | | | |
| TAC | CTG | GCC | TCC | GTC | AAA | GCC | ATG | CAC | GAG | GCT | TCC | AAG | AAG | CTG | AAT | 299 |
| Tyr | Leu | Ala | Ser | Val | Lys | Ala | Met | His | Glu | Ala | Ser | Lys | Lys | Leu | Asn | |
| | 65 | | | | 70 | | | | 75 | | | | | 80 | | |
| GAG | TGT | CTG | CAG | GAG | GTG | TAT | GAG | CCC | GAT | TGG | CCC | GGC | AGG | GAT | GAG | 347 |
| Glu | Cys | Leu | Gln | Glu | Val | Tyr | Glu | Pro | Asp | Trp | Pro | Gly | Arg | Asp | Glu | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| GCA | AAC | AAG | ATC | GCA | GAG | AAC | AAC | GAC | CTG | CTG | TGG | ATG | GAT | TAC | CAC | 395 |
| Ala | Asn | Lys | Ile | Ala | Glu | Asn | Asn | Asp | Leu | Leu | Trp | Met | Asp | Tyr | His | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| CAG | AAG | CTG | GTG | GAC | CAG | GCG | CTG | CTG | ACC | ATG | GAC | ACG | TAC | CTG | GGC | 443 |
| Gln | Lys | Leu | Val | Asp | Gln | Ala | Leu | Leu | Thr | Met | Asp | Thr | Tyr | Leu | Gly | |
| | | 115 | | | | | 120 | | | | | 125 | | | | |
| CAG | TTC | CCC | GAC | ATC | AAG | TCA | CGC | ATT | GCC | AAG | CGG | GGG | CGC | AAG | CTG | 491 |
| Gln | Phe | Pro | Asp | Ile | Lys | Ser | Arg | Ile | Ala | Lys | Arg | Gly | Arg | Lys | Leu | |
| | 130 | | | | | 135 | | | | | 140 | | | | | |
| GTG | GAC | TAC | GAC | AGT | GCC | CGG | CAC | CAC | TAC | GAG | TCC | CTT | CAA | ACT | GCC | 539 |
| Val | Asp | Tyr | Asp | Ser | Ala | Arg | His | His | Tyr | Glu | Ser | Leu | Gln | Thr | Ala | |
| | 145 | | | | 150 | | | | | 155 | | | | | 160 | |
| AAA | AAG | AAG | GAT | GAA | GCC | AAA | ATT | GCC | AAG | GCC | GAG | GAG | GAG | CTC | ATC | 587 |
| Lys | Lys | Lys | Asp | Glu | Ala | Lys | Ile | Ala | Lys | Ala | Glu | Glu | Glu | Leu | Ile | |
| | | | 165 | | | | | 170 | | | | | | 175 | | |
| AAA | GCC | CAG | AAG | GTG | TTT | GAG | GAG | ATG | AAT | GTG | GAT | CTG | CAG | GAG | GAG | 635 |
| Lys | Ala | Gln | Lys | Val | Phe | Glu | Glu | Met | Asn | Val | Asp | Leu | Gln | Glu | Glu | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| CTG | CCG | TCC | CTG | TGG | AAC | AGC | CGC | GTA | GGT | TTC | TAC | GTC | AAC | ACG | TTC | 683 |
| Leu | Pro | Ser | Leu | Trp | Asn | Ser | Arg | Val | Gly | Phe | Tyr | Val | Asn | Thr | Phe | |
| | | 195 | | | | | 200 | | | | | 205 | | | | |

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| | |
|---|------|
| CAG AGC ATC GCG GGC CTG GAG GAA AAC TTC CAC AAG GAG ATG AGC AAG Gln Ser Ile Ala Gly Leu Glu Glu Asn Phe His Lys Glu Met Ser Lys 210 215 220 | 731 |
| CTC AAC CAG AAC CTC AAT GAT GTG CTG GTC GGC CTG GAG AAG CAA CAC Leu Asn Gln Asn Leu Asn Asp Val Leu Val Gly Leu Glu Lys Gln His 225 230 235 240 | 779 |
| GGG AGC AAC ACC TTC ACG GTC AAG GCC CAG CCC AGA AAG AAA AGT AAA Gly Ser Asn Thr Phe Thr Val Lys Ala Gln Pro Arg Lys Lys Ser Lys 245 250 255 | 827 |
| CTG TTT TCG CGG CTG CGC AGA AAG AAG AAC AGT GAC AAC GCG CCT GCA Leu Phe Ser Arg Leu Arg Arg Lys Lys Asn Ser Asp Asn Ala Pro Ala 260 265 270 | 875 |
| AAA GGG AAC AAG AGC CCT TCG CCT CCA GAT GGC TCC CCT GCC GCC ACC Lys Gly Asn Lys Ser Pro Ser Pro Pro Asp Gly Ser Pro Ala Ala Thr 275 280 285 | 923 |
| CCC GAG ATC AGA GTC AAC CAC GAG CCA GAG CCG GCC GGC GGG GCC ACG Pro Glu Ile Arg Val Asn His Glu Pro Glu Pro Ala Gly Gly Ala Thr 290 295 300 | 971 |
| CCC GGG GCC ACC CTC CCC AAG TCC CCA TCT CAG CCA GCA GAG GCC TCG Pro Gly Ala Thr Leu Pro Lys Ser Pro Ser Gln Pro Ala Glu Ala Ser 305 310 315 320 | 1019 |
| GAG GTG GCG GGT GGG ACC CAA CCT GCG GCT GGA GCC CAG GAG CCA GGG Glu Val Ala Gly Gly Thr Gln Pro Ala Ala Gly Ala Gln Glu Pro Gly 325 330 335 | 1067 |
| GAG ACT TCT GCA AGT GAA GCA GCC TCC AGC TCT CTT CCT GCT GTC GTG Glu Thr Ser Ala Ser Glu Ala Ala Ser Ser Ser Leu Pro Ala Val Val 340 345 350 | 1115 |
| GTG GAG ACC TTC CCA GCA ACT GTG AAT GGC ACC GTG GAG GGC GGC AGT Val Glu Thr Phe Pro Ala Thr Val Asn Gly Thr Val Glu Gly Gly Ser 355 360 365 | 1163 |
| GGG GCC GGG CGC TTG GAC CTG CCC CCA GGT TTC ATG TTC AAG GTA CAG Gly Ala Gly Arg Leu Asp Leu Pro Pro Gly Phe Met Phe Lys Val Gln 370 375 380 | 1211 |
| GCC CAG CAC GAC TAC ACG GCC ACT GAC ACA GAG GAG CTG CAG CTC AAG Ala Gln His Asp Tyr Thr Ala Thr Asp Thr Asp Glu Leu Gln Leu Lys 385 390 395 400 | 1259 |
| GCT GGT GAT GTG GTG CTG GTG ATC CCC TTC CAG AAC CCT GAA GAG CAG Ala Gly Asp Val Val Leu Val Ile Pro Phe Gln Asn Pro Glu Glu Gln 405 410 415 | 1307 |
| GAT GAA GGC TGG CTC ATG GGC GTG AAG GAG AGC GAC TGG AAC CAG CAC Asp Glu Gly Trp Leu Met Gly Val Lys Glu Ser Asp Trp Asn Gln His 420 425 430 | 1355 |
| AAG AAG CTG GAG AAG TGC CGT GGC GTC TTC CCC GAG AAC TTC ACT GAG Lys Lys Leu Glu Lys Cys Arg Gly Val Phe Pro Glu Asn Phe Thr Glu 435 440 445 | 1403 |
| AGG GTC CCA TGACGGCGGG GCCCAGGCAG CCTCCGGGCG TGTGAAGAAC Arg Val Pro 450 | 1452 |

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ACCTCCTCCC GAAAAATGTG TGGTCTTTTT TTTTGTTTTG TTTTCGTTTT TCATCTTTTG 1512
 AAGAGCAAAG GGAAATCAAG AGGAGACCCC CAGGCAGAGG GGCCTTCTCC CAAAGTTTAG 1572
 GTCGTTTTCC AAAGAGCCGC GTCCCGGCAA GTCCGGCGGA ATTCACCACT GTTCCTGAAG 1632
 CTGCTGTGTC CTCTAGTTGA GTTTCTGGCG CCCCTGCCTG TGCCCGCATG TGTGCCTGGC 1692
 CGCAGGGCGG GGCTGGGGGC TGCCGAGCCA CCATACTTAA CTGAAGCTTC GGCCGCACCA 1752
 CCCGGGGAAG GGTCCTCTTT TCCTGGCAGC TGCTGTGGGT GGGGCCCAGA CACCAGCCTA 1812
 GCCTGCTCTG CCCCGCAGAC GGTCTGTGTG CTGTTTGAAA ATAAATCTTA GTGTTCAAAA 1872
 CAAATGAAA CAAAAAATAA AATGATAAAA ACTCTCAAAA AAACAAGGAA TTC 1925

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 451 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Leu Trp Asn Val Val Thr Ala Gly Lys Ile Ala Ser Asn Val Gln
 1 5 10 15
 Lys Lys Leu Thr Arg Ala Gln Glu Lys Val Leu Gln Lys Leu Gly Lys
 20 25 30
 Ala Asp Glu Thr Lys Asp Glu Gln Phe Glu Gln Cys Val Gln Asn Phe
 35 40 45
 Asn Lys Gln Leu Thr Glu Gly Thr Arg Leu Gln Lys Asp Leu Arg Thr
 50 55 60
 Tyr Leu Ala Ser Val Lys Ala Met His Glu Ala Ser Lys Lys Leu Asn
 65 70 75 80
 Glu Cys Leu Gln Glu Val Tyr Glu Pro Asp Trp Pro Gly Arg Asp Glu
 85 90 95
 Ala Asn Lys Ile Ala Glu Asn Asn Asp Leu Leu Trp Met Asp Tyr His
 100 105 110
 Gln Lys Leu Val Asp Gln Ala Leu Leu Thr Met Asp Thr Tyr Leu Gly
 115 120 125
 Gln Phe Pro Asp Ile Lys Ser Arg Ile Ala Lys Arg Gly Arg Lys Leu
 130 135 140
 Val Asp Tyr Asp Ser Ala Arg His His Tyr Glu Ser Leu Gln Thr Ala
 145 150 155 160
 Lys Lys Lys Asp Glu Ala Lys Ile Ala Lys Ala Glu Glu Glu Leu Ile
 165 170 175
 Lys Ala Gln Lys Val Phe Glu Glu Met Asn Val Asp Leu Gln Glu Glu
 180 185 190

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[illegible]

(2) INFORMATION FOR SEQ ID NO:7:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(11) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Leu Xaa Gly Xaa Glu
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Xaa Cys Xaa Glu
1 5

WHAT IS CLAIMED IS:

1. A mammalian nucleic acid sequence encoding a BIN1-Associated U1-specific protein (Bau) or a fragment thereof, isolated from cellular materials with which it is naturally associated, selected from the group consisting of:

- (a) SEQ ID NO:1;
- (b) a sequence which hybridizes to (a) under stringent conditions;
- (c) an allelic variation of (a); and
- (d) a fragment of (a) of at least 15 nucleotides.

2. The sequence according to claim 1 which encodes murine Bau SEQ ID NO:2 or a fragment thereof.

3. The sequence according to claim 1, wherein the Bau fragment is selected from the group consisting of:

- (a) nt 231 to 674 of SEQ ID NO:1;
- (b) nt 48 to 527 of SEQ ID NO:1;
- (c) nt 312 to 362 of SEQ ID NO:1;
- (d) nt 294 to 356 of SEQ ID NO: 1; and
- (e) nt 540 to 554 of SEQ ID NO:1.

4. A murine cDNA sequence SEQ ID NO:1 which encodes a Bau polypeptide.

5. A mammalian BIN1-Associated U1-specific protein (Bau) polypeptide, said polypeptide selected from the group consisting of:

- (a) murine Bau, SEQ ID NO:2;
- (b) human Bau;
- (c) a fragment of (a) or (b) having biological activity; and

(d) analogues or homologs of (a) or (b) characterized by having at least 85% homology with SEQ ID NO: 2.

6. The Bau polypeptide according to claim 5, wherein the fragment is selected from the group consisting of:

- (a) aa 62 to 209 of SEQ ID NO:2;
- (b) aa 1 to 160 of SEQ ID NO:2;
- (c) aa 89 to 105 of SEQ ID NO:2;
- (d) aa 83 to 103 of SEQ ID NO:2; and
- (e) aa 165 to 169 of SEQ ID NO:2.

7. A vector comprising a mammalian nucleic acid sequence encoding a BIN1-Associated U1-specific protein (Bau) polypeptide under the control of suitable regulatory sequences, said polypeptide selected from the group consisting of:

- (a) murine Bau SEQ ID NO:2;
- (b) human Bau;
- (c) a fragment of (a) or (b) having biological activity; and
- (d) analogues or homologs of (a) or (b) characterized by having at least 90% homology with SEQ ID NO: 2.

8. A host cell transformed with the vector according to claim 7.

9. An oligonucleotide probe comprising a nucleic acid sequence selected from the group consisting of:

- (a) SEQ ID NO:1;
- (b) a nucleic acid fragment of (a) comprising at least 15 nucleotides in length,

and a detectable label which is associated with said sequence.

10. An anti-BIN1-Associated U1-specific protein (Bau) antibody.

11. The anti-Bau antibody according to claim 10 directed against a Bau peptide, said peptide selected from the group consisting of:

- (a) SEQ ID NO:2; and
- (b) a fragment of (a) comprising about 8 amino acids.

12. The antibody according to claim 10, selected from the group consisting of a chimeric antibody, a humanized antibody, a monoclonal antibody and a polyclonal antibody.

13. An anti-idiotypic antibody specific for the antibody of claim 10.

14. A diagnostic reagent comprising the antibody according to claim 10 and a detectable label.

15. A pharmaceutical composition comprising the vector according to claim 7 and a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising the anti-idiotypic antibody according to claim 13 and a pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a mammalian BIN1-Associated U1-specific protein (Bau) polypeptide selected from the group consisting of:

- (a) SEQ ID NO:2; and
- (b) a fragment of (a) having biological activity.

18. A method of detecting a cancer involving the c-MYC oncogene or a hyperplastic disease state comprising providing a biopsy sample from a patient suspected of having said cancer or disease and incubating said sample in the presence of a diagnostic reagent according to claim 14.

19. A method of detecting a cancer involving the c-MYC oncogene or a hyperplastic disease state comprising providing a biopsy sample from a patient suspected of having said cancer or disease and performing the polymerase chain reaction using the oligonucleotide probe according to claim 13.

20. A method of detecting a deficiency in Bau in a patient comprising providing a sample from a patient suspected of having said deficiency and incubating said sample in the presence of a diagnostic reagent according to claim 14.

21. A method of detecting a deficiency in Bau in a patient comprising providing a sample from a patient suspected of having said deficiency and performing the polymerase chain reaction using the oligonucleotide probe according to claim 13.

22. A method of treating deficiencies in Bau in a patient comprising administering to said patient a pharmaceutical composition according to claim 17.

23. A method of treating a cancer or hyperplastic disease state involving the c-MYC oncogene comprising administering to a patient having said cancer or disease a pharmaceutical composition according to claim 17.

24. A method of identifying compounds which specifically bind to Bau or a fragment thereof, comprising the steps of contacting said Bau or fragment with a test compound to permit binding of the test compound to Bau; and determining the amount of test compound which is bound to Bau.

25. A compound identified by the method of claim 24.

26. Use of a Bau nucleic acid according to claim 1, a Bau polypeptide according to claim 5, or an anti-idiotypic antibody according to claim 13 in the preparation of a medicament.

27. Use of an anti-Bau antibody according to claim 10 in the preparation of a medicament.

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FIGURE 1A

| | | | | | | | |
|------------|------------|------------|------------|---------|-----|-----|-----|
| GGGGTGACGT | GGCCATTGAG | GTCTTTGAGC | TGCCTGAGAA | CGAGGAC | ATG | TTT | 53 |
| | | | | | Met | Phe | |
| | | | | | 1 | | |
| TCC | CCA | TCT | GAC | CTG | GAC | ACA | 98 |
| Ser | Pro | Ser | Asp | Leu | Asp | Thr | |
| | | 5 | | | | 10 | |
| | | | | | | 15 | |
| GAG | TTG | CAA | ATC | AAA | CAT | GCA | 143 |
| Glu | Leu | Gln | Ile | Lys | His | Ala | |
| | | 20 | | | | 25 | |
| | | | | | | 30 | |
| TTG | AAG | ACC | AAG | CTT | CAA | GCA | 188 |
| Leu | Lys | Thr | Lys | Leu | Gln | Ala | |
| | | 35 | | | | 40 | |
| | | | | | | 45 | |
| GAA | CTA | GAA | AAG | AAC | CAA | CTG | 233 |
| Glu | Leu | Glu | Lys | Asn | Gln | Leu | |
| | | 50 | | | | 55 | |
| | | | | | | 60 | |
| GAA | CGG | ATG | CTG | AAG | TTG | GAG | 278 |
| Glu | Arg | Met | Leu | Lys | Leu | Glu | |
| | | 65 | | | | 70 | |
| | | | | | | 75 | |
| TTA | TGT | CAT | ACG | GTG | AAT | GAG | 323 |
| Leu | Cys | His | Thr | Val | Asn | Glu | |
| | | 80 | | | | 85 | |
| | | | | | | 90 | |
| TAC | CAA | GCC | CTG | GAA | AAG | AAA | 368 |
| Tyr | Gln | Ala | Leu | Glu | Lys | Lys | |
| | | 95 | | | | 100 | |
| | | | | | | 105 | |
| AAA | GAC | TTC | CAG | CAA | AAA | GAG | 413 |
| Lys | Asp | Phe | Gln | Gln | Lys | Glu | |
| | | 110 | | | | 115 | |
| | | | | | | 120 | |
| GTA | GAA | AGA | AAG | AAG | CGG | GAG | 458 |
| Val | Glu | Arg | Lys | Lys | Arg | Glu | |
| | | 125 | | | | 130 | |
| | | | | | | 135 | |
| GAA | GTC | CAA | GGC | CTG | CAA | GTT | 503 |
| Glu | Val | Gln | Gly | Leu | Gln | Val | |
| | | 140 | | | | 145 | |
| | | | | | | 150 | |
| GTG | TTC | AGA | CTA | CTA | AAG | CAA | 548 |
| Val | Phe | Arg | Leu | Leu | Lys | Gln | |
| | | 155 | | | | 160 | |
| | | | | | | 165 | |

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FIGURE 1B

[illegible]

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FIGURE 2

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GAG | ATC | AGA | GTG | AAC | CAT | GAG | CCA | GAG | CCG | GCC | AGT | GGG | GCC | TCA | 45 |
| Glu | Ile | Arg | Val | Asn | His | Glu | Pro | Glu | Pro | Ala | Ser | Gly | Ala | Ser | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| CCC | GGG | GCT | GCC | ATC | CCC | AAG | TCC | CCA | TCT | CAG | CCA | GCA | GAG | GCC | 90 |
| Pro | Gly | Ala | Ala | Ile | Pro | Lys | Ser | Pro | Ser | Gln | Pro | Ala | Glu | Ala | |
| | | | | 20 | | | | | 25 | | | | | 30 | |
| TCC | GAG | GTG | GTG | GGT | GGA | GCC | CAG | GAG | CCA | GGG | GAG | ACA | GCA | GCC | 135 |
| Ser | Glu | Val | Val | Gly | Gly | Ala | Gln | Glu | Pro | Gly | Glu | Thr | Ala | Ala | |
| | | | | 35 | | | | | 40 | | | | | 45 | |
| AGT | GAA | GCA | ACC | TCC | AGC | TCT | CTT | CCG | GCT | GTG | GTG | GTG | GAG | ACC | 180 |
| Ser | Glu | Ala | Thr | Ser | Ser | Ser | Leu | Pro | Ala | Val | Val | Val | Glu | Thr | |
| | | | | 50 | | | | | 55 | | | | | 60 | |
| TTC | TCC | GCA | ACT | GTG | AAT | GGG | GCG | GTG | GAG | GGC | AGC | GCT | GGG | ACT | 225 |
| Phe | Ser | Ala | Thr | Val | Asn | Gly | Ala | Val | Glu | Gly | Ser | Ala | Gly | Thr | |
| | | | | 65 | | | | | 70 | | | | | 75 | |
| GGA | CGC | TTG | GAC | CTG | CCC | CCG | GGA | TTC | ATG | TTC | AAG | GTT | CAA | GCC | 270 |
| Gly | Arg | Leu | Asp | Leu | Pro | Pro | Gly | Phe | Met | Phe | Lys | Val | Gln | Ala | |
| | | | | 80 | | | | | 85 | | | | | 90 | |
| CAG | CAT | GAT | TAC | ACG | GCC | ACT | GAC | ACT | GAT | GAG | CTG | CAA | CTC | AAA | 315 |
| Gln | His | Asp | Tyr | Thr | Ala | Thr | Asp | Thr | Asp | Glu | Leu | Gln | Leu | Lys | |
| | | | | 95 | | | | | 100 | | | | | 105 | |
| GCT | GGC | GAT | GTG | GTG | TTG | GTG | ATT | CCT | TTC | CAG | AAC | CCA | GAG | GAG | 360 |
| Ala | Gly | Asp | Val | Val | Leu | Val | Ile | Pro | Phe | Gln | Asn | Pro | Glu | Glu | |
| | | | | 110 | | | | | 115 | | | | | 120 | |
| CAG | GAT | GAA | GGC | TGG | CTC | ATG | GGT | GTG | AAG | GAG | AGC | GAC | TGA | | 402 |
| Gln | Asp | Glu | Gly | Trp | Leu | Met | Gly | Val | Lys | Glu | Ser | Asp | | | |
| | | | | 125 | | | | | 130 | | | | | | |

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FIGURE 3A

| | |
|---|-----|
| GAATTCCGTG CTGGTTGAGC TTGCTCATCT CCTTGTGGAA GTTTTCCTCC | 50 |
| AGGCCCCGCG ATG CTC TGG AAC GTG GTG ACG GCG GGA AAG ATC GCC | 95 |
| Met Leu Trp Asn Val Val Thr Ala Gly Lys Ile Ala | |
| 1 5 10 | |
| AGC AAC GTG CAG AAG AAG CTC ACC CGC GCG CAG GAG AAG GTT CTC | 140 |
| Ser Asn Val Gln Lys Lys Leu Thr Arg Ala Gln Glu Lys Val Leu | |
| 15 20 25 | |
| CAG AAG CTG GGG AAG GCA GAT GAG ACC AAG GAT GAG CAG TTT GAG | 185 |
| Gln Lys Leu Gly Lys Ala Asp Glu Thr Lys Asp Glu Gln Phe Glu | |
| 30 35 40 | |
| CAG TGC GTC CAG AAT TTC AAC AAG CAG CTG ACG GAG GGC ACC CGG | 230 |
| Gln Cys Val Gln Asn Phe Asn Lys Gln Leu Thr Glu Gly Thr Arg | |
| 45 50 55 | |
| CTG CAG AAG GAT CTC CGG ACC TAC CTG GCC TCC GTC AAA GCC ATG | 275 |
| Leu Gln Lys Asp Leu Arg Thr Tyr Leu Ala Ser Val Lys Ala Met | |
| 60 65 70 | |
| CAC GAG GCT TCC AAG AAG CTG AAT GAG TGT CTG CAG GAG GTG TAT | 320 |
| His Glu Ala Ser Lys Lys Leu Asn Glu Cys Leu Gln Glu Val Tyr | |
| 75 80 85 | |
| GAG CCC GAT TGG CCC GGC AGG GAT GAG GCA AAC AAG ATC GCA GAG | 365 |
| Glu Pro Asp Trp Pro Gly Arg Asp Glu Ala Asn Lys Ile Ala Glu | |
| 90 95 100 | |
| AAC AAC GAC CTG CTG TGG ATG GAT TAC CAC CAG AAG CTG GTG GAC | 410 |
| Asn Asn Asp Leu Leu Trp Met Asp Tyr His Gln Lys Leu Val Asp | |
| 105 110 115 | |
| CAG GCG CTG CTG ACC ATG GAC ACG TAC CTG GGC CAG TTC CCC GAC | 455 |
| Gln Ala Leu Leu Thr Met Asp Thr Tyr Leu Gly Gln Phe Pro Asp | |
| 120 125 130 | |
| ATC AAG TCA CGC ATT GCC AAG CGG GGG CGC AAG CTG GTG GAC TAC | 500 |
| Ile Lys Ser Arg Ile Ala Lys Arg Gly Arg Lys Leu Val Asp Tyr | |
| 135 140 145 | |
| GAC AGT GCC CGG CAC CAC TAC GAG TCC CTT CAA ACT GCC AAA AAG | 545 |
| Asp Ser Ala Arg His His Tyr Glu Ser Leu Gln Thr Ala Lys Lys | |
| 150 155 160 | |
| AAG GAT GAA GCC AAA ATT GCC AAG GCC GAG GAG GAG CTC ATC AAA | 590 |
| Lys Asp Glu Ala Lys Ile Ala Lys Ala Glu Glu Glu Leu Ile Lys | |
| 165 170 175 | |

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FIGURE 3B

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| GCC | CAG | AAG | GTG | TTT | GAG | GAG | ATG | AAT | GTG | GAT | CTG | CAG | GAG | GAG | 635 |
| Ala | Gln | Lys | Val | Phe | Glu | Glu | Met | Asn | Val | Asp | Leu | Gln | Glu | Glu | |
| | | 180 | | | | | 185 | | | | | 190 | | | |
| CTG | CCG | TCC | CTG | TGG | AAC | AGC | CGC | GTA | GGT | TTC | TAC | GTC | AAC | ACG | 680 |
| Leu | Pro | Ser | Leu | Trp | Asn | Ser | Arg | Val | Gly | Phe | Tyr | Val | Asn | Thr | |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| TTC | CAG | AGC | ATC | GCG | GGC | CTG | GAG | GAA | AAC | TTC | CAC | AAG | GAG | ATG | 725 |
| Phe | Gln | Ser | Ile | Ala | Gly | Leu | Glu | Glu | Asn | Phe | His | Lys | Glu | Met | |
| | | 210 | | | | | 215 | | | | | 220 | | | |
| AGC | AAG | CTC | AAC | CAG | AAC | CTC | AAT | GAT | GTG | CTG | GTC | GGC | CTG | GAG | 770 |
| Ser | Lys | Leu | Asn | Gln | Asn | Leu | Asn | Asp | Val | Leu | Val | Gly | Leu | Glu | |
| | | 225 | | | | | 230 | | | | | 235 | | | |
| AAG | CAA | CAC | GGG | AGC | AAC | ACC | TTC | ACG | GTC | AAG | GCC | CAG | CCC | AGA | 815 |
| Lys | Gln | His | Gly | Ser | Asn | Thr | Phe | Thr | Val | Lys | Ala | Gln | Pro | Arg | |
| | | 240 | | | | | 245 | | | | | 250 | | | |
| AAG | AAA | AGT | AAA | CTG | TTT | TCG | CGG | CTG | CGC | AGA | AAG | AAG | AAC | AGT | 860 |
| Lys | Lys | Ser | Lys | Leu | Phe | Ser | Arg | Leu | Arg | Arg | Lys | Lys | Asn | Ser | |
| | | 255 | | | | | 260 | | | | | 265 | | | |
| GAC | AAC | GCG | CCT | GCA | AAA | GGG | AAC | AAG | AGC | CCT | TCG | CCT | CCA | GAT | 905 |
| Asp | Asn | Ala | Pro | Ala | Lys | Gly | Asn | Lys | Ser | Pro | Ser | Pro | Pro | Asp | |
| | | 270 | | | | | 275 | | | | | 280 | | | |
| GGC | TCC | CCT | GCC | GCC | ACC | CCC | GAG | ATC | AGA | GTC | AAC | CAC | GAG | CCA | 950 |
| Gly | Ser | Pro | Ala | Ala | Thr | Pro | Glu | Ile | Arg | Val | Asn | His | Glu | Pro | |
| | | 285 | | | | | 290 | | | | | 295 | | | |
| GAG | CCG | GCC | GGC | GGG | GCC | ACG | CCC | GGG | GCC | ACC | CTC | CCC | AAG | TCC | 995 |
| Glu | Pro | Ala | Gly | Gly | Ala | Thr | Pro | Gly | Ala | Thr | Leu | Pro | Lys | Ser | |
| | | 300 | | | | | 305 | | | | | 310 | | | |
| CCA | TCT | CAG | CCA | GCA | GAG | GCC | TCG | GAG | GTG | GCG | GGT | GGG | ACC | CAA | 1040 |
| Pro | Ser | Gln | Pro | Ala | Glu | Ala | Ser | Glu | Val | Ala | Gly | Gly | Thr | Gln | |
| | | 315 | | | | | 320 | | | | | 325 | | | |
| CCT | GCG | GCT | GGA | GCC | CAG | GAG | CCA | GGG | GAG | ACT | TCT | GCA | AGT | GAA | 1085 |
| Pro | Ala | Ala | Gly | Ala | Gln | Glu | Pro | Gly | Glu | Thr | Ser | GCA | Ala | Ser | |
| | | 330 | | | | | 335 | | | | | 340 | | | |
| GCA | GCC | TCC | AGC | TCT | CTT | CCT | GCT | GTC | GTG | GTG | GAG | ACC | TTC | CCA | 1130 |
| Ala | Ala | Ser | Ser | Ser | Leu | Pro | Ala | Val | Val | Val | Glu | Thr | Phe | Pro | |
| | | 345 | | | | | 350 | | | | | 355 | | | |

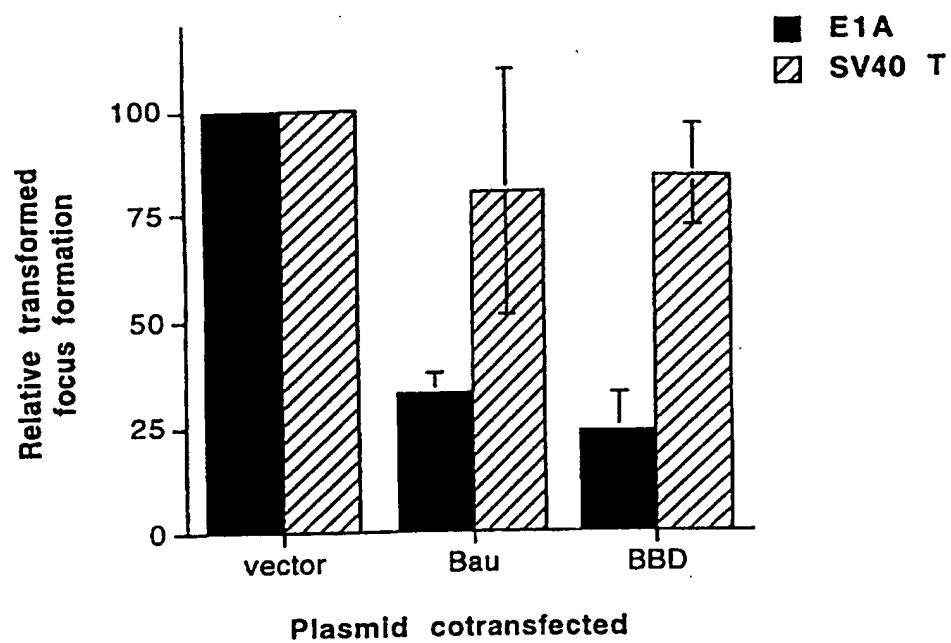
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FIGURE 3C

| | |
|---|------|
| GCA ACT GTG AAT GGC ACC GTG GAG GGC GGC AGT GGG GCC GGG CGC | 1175 |
| Ala Thr Val Asn Gly Thr Val Glu Gly Gly Ser Gly Ala Gly Arg | |
| 360 365 370 | |
| TTG GAC CTG CCC CCA GGT TTC ATG TTC AAG GTA CAG GCC CAG CAC | 1220 |
| Leu Asp Leu Pro Pro Gly Phe Met Phe Lys Val Gln Ala Gln His | |
| 375 380 385 | |
| GAC TAC ACG GCC ACT GAC ACA GAC GAG CTG CAG CTC AAG GCT GGT | 1265 |
| Asp Tyr Thr Ala Thr Asp Thr Asp Glu Leu Gln Leu Lys Ala Gly | |
| 390 395 400 | |
| GAT GTG GTG CTG GTG ATC CCC TTC CAG AAC CCT GAA GAG CAG GAT | 1310 |
| Asp Val Val Leu Val Ile Pro Phe Gln Asn Pro Glu Glu Gln Asp | |
| 405 410 415 | |
| GAA GGC TGG CTC ATG GGC GTG AAG GAG AGC GAC TGG AAC CAG CAC | 1355 |
| Glu Gly Trp Leu Met Gly Val Lys Glu Ser Asp Trp Asn Gln His | |
| 420 425 430 | |
| AAG AAG CTG GAG AAG TGC CGT GGC GTC TTC CCC GAG AAC TTC ACT | 1400 |
| Lys Lys Leu Glu Lys Cys Arg Gly Val Phe Pro Glu Asn Phe Thr | |
| 435 440 445 | |
| GAG AGG GTC CCA TGACGGCGGG GCCCAGGCAG CCTCCGGGCG TGTGAAGAAC | 1452 |
| Glu Arg Val Pro | |
| 450 | |
| ACCTCCTCCC GAAAAATGTG TGGTTCTTTT TTTTGTTTTG TTTTCGTTTT | 1502 |
| TCATCTTTTG AAGAGCAAAG GGAAATCAAG AGGAGACCCC CAGGCAGAGG | 1552 |
| GGCGTTCTCC CAAAGTTTAG GTCGTTTTCC AAAGAGCCGC GTCCCGGCAA | 1602 |
| GTCCGGCGGA ATTCACCAGT GTTCCTGAAG CTGCTGTGTC CTCTAGTTGA | 1652 |
| GTTTCTGGCG CCCCTGCCTG TGCCCGCATG TGTGCCTGGC CGCAGGGCGG | 1702 |
| GGCTGGGGGC TGCCGAGCCA CCATACTTAA CTGAAGCTTC GGCCGCACCA | 1752 |
| CCCGGGGAAG GGTCTCTTTT TCCTGGCAGC TGCTGTGGGT GGGGCCCAGA | 1802 |
| CACCAGCCTA GCCTGCTCTG CCCCAGCAGAC GGTCTGTGTG CTGTTTGAAA | 1852 |
| ATAAATCTTA GTGTTCAAAA CAAAATGAAA CAAAAAATAA AATGATAAAA | 1902 |
| ACTCTCAAAA AAACAAGGAA TTC | 1925 |

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FIGURE 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/15298

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet

US CL : 536/23.5; 530/350, 387.1; 435/6, 7.1, 252.3, 325; 514/2, 44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 530/350, 387.1; 435/6, 7.1, 252.3, 325; 514/2, 44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MPSRCII

search terms: BIN1-associated U1-specific protein, Bau

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|--------------|--|------------------------------------|
| X -- Y | Database on MPSRCH, Genbank, Accession No. G24494. 'Human STSs derived from sequences in dbEST and the Unigene collection'. HUDSON, T. 1995. See attached MPSRCH for similar nucleotides, from nucleotides numbers 301 to 324. | 1-3, 17 ----- 7-9, 15, 18-21 |
| X -- Y | WO 84/04537 A1, (BATELLE INSTITUTE), 22 November 1984. See especially attached MPSRCH for similar nucleotides, from nucleotides numbers 540 to 554. | 1-3, 17 ----- 7-9, 15, 18-21 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *B* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *A* document member of the same patent family |
| *O* document referring to an oral disclosure, use, exhibition or other means | |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

21 OCTOBER 1997

Date of mailing of the international search report

13 JAN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15298

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 15, 17-21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15298

A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C07H 21/04; C07K 14/00, 16/00; C12N 15/85; G01N 33/53; C12Q 1/68; A61K 38/00, 48/00

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-9, 15, 17-21, drawn to 1) a mammalian BIN1-associated U1-specific protein (Bau), functional fragments or analogues, or homologs thereof, 2) its encoding nucleotide sequences, or an allelic variant, or a fragment of at least 15 nucleotides thereof, or a sequence hybridizing to said Bau nucleotide sequence, 3) a vector comprising said Bau nucleotide sequence, 4) a host cell transformed with said vector, 5) an oligonucleotide probe comprising said nucleotide sequence, or fragments thereof, 6) a pharmaceutical composition comprising said vector, or said Bau polypeptide, or fragments of said Bau polypeptide, and a method of detection of cancer or detection of a deficiency in Bau.

Group II, claim(s) 10-14, 16, drawn to antibodies against a Bau peptide or its fragment, or anti-idiotypic antibodies specific for said antibodies, a diagnostic reagent, and a pharmaceutical composition comprising said antibodies.

Group III, claim(s) 22-23, 26-27, drawn to a method of treating cancer or deficiencies in Bau.

Group IV, claim(s) 24-25, drawn to a method of identifying compounds which specifically bind to Bau or fragments thereof, and a compound identified by said method.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
An international stage application shall relate to one invention only or to a group of invention so linked as to form a single general inventive concept. If multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application will be considered as the main invention in the claims, see PCT article 17(3) (a) and 1.476 (c). Group I will be the main invention.

Group I, claims 1-9, 15, 17-21 form a single inventive concept. Group II is an additional product, an antibody against Bau polypeptide, or its fragments, and groups III-IV are additional use claimed for said antibodies, or for said Bau nucleotide sequence, or its fragments.